

Protocol for evaluation of highly diluted *Atropa belladonna* in murine infection by *Trypanosoma cruzi*

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Background: The Multicentre International Project on High Dilutions suggests evaluating the effect of the compound *A. belladonna* in different experimental models.

Aim: The aim is to evaluate the effect of *A. belladonna* ultra-diluted in the course of murine infection caused by *T. cruzi*.

Methodology: The experiments, approved by the UEM's Animal Ethics Committee, will be performed as a blind controlled, randomized assay by chance. Animals will be maintained in an environment with temperature (18-20 °C), humidity (70%) and lighting controlled, in a 12 hours light and dark cycle, and will receive water and food *ad libitum* in micro-acclimated cages. Provided by the UEM's Central Animal Vivarium, swiss male mice, 56 days old were divided in groups of 12 animals each according to the treatment: CI- infected untreated; CNI- not infected and not treated; and animals infected and treated with the TM - mother tincture of *A. belladonna* and their dynamized solutions: 2cH; 3cH; 5cH; 6cH; 12cH and 30cH.

Infection: 1400 blood trypomastigotes of the Y strain of *T. cruzi*, *via i.p.*

Treatment: Will be provided *ad libitum* diluted in water (1mL/100mL water) 48 hours before and after the infection, subsequently in 56/56 hours until complete nine days of infection. It will be available to the animals during 16 hours. Dynamizations will be prepared from the *A. belladonna* TM (commercially purchased) according to the Brazilian Homeopathic Pharmacopoeia, with microbiological testing (RDC67-MS Brazil) and *in vivobiological* test.

Analyses: Clinical parameters (temperature, weight, water/food intake and excreta) [1,2] and parasitological parameters (pre-patent, patent period, parasitemia peak, total parasitemia, mortality and survival time) will be assessed daily. The dynamization that presents the best performance will be elected for behavioral study through the automated system NoldusEthoVision XT6 (Noldus, Leesburg, Netherlands). The analysis will include assessment of motor activity (distance covered in the arena and average speed of each animal), and

the exploratory activity (frequency of events and time spent by the animal in fields determined by the software).

Considerations: *In vivo* experiments using highly/ultra diluted substances demonstrate that they are able to influence the clinical with direct reflects in the animal's behavioral equilibrium [3]. The *A. belladonna* is a plant rich in primary alkaloids [4] and it is used as a modulator of inflammatory processes [5,6].

Keywords: *Trypanosoma cruzi*, High dilutions, *Atropa belladonna*, *Murine Infecçion*

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